(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 350 205 B1**

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **02.08.95** (51) Int. Cl.6: **C12Q 1/68**

(21) Application number: **89306594.6**

(22) Date of filing: **29.06.89**

(54) **Detection of campylobacter.**

(30) Priority: **07.07.88 US 216679**

(43) Date of publication of application:
10.01.90 Bulletin 90/02

(45) Publication of the grant of the patent:
02.08.95 Bulletin 95/31

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(56) References cited:
EP-A- 0 232 085
EP-A- 0 272 009
WO-A-86/04422

FEMS MICROBIOLOGY LETTERS, vol. 43, 1987, Amsterdam (NL); P.J. ROMANIUK et al., pp. 331-335&NUM;

INTERNATL. JOURNAL OF SYSTEMATIC BACTERIOLOGY, vol. 38, no. 2, April 1988, Ames (US); L.M. THOMPSON et al., pp. 190-200&NUM;

INTERNATL. JOURNAL OF SYSTEMATIC BACTERIOLOGY, vol. 38, no. 1, January 1988, Ames (US); B.J. PASTER et al., pp. 56-62&NUM;

(73) Proprietor: **AMOCO CORPORATION**
Mail Code 1907, P.O. Box 87703
Chicago, IL 60680-0703 (US)

(72) Inventor: **Barns, Susan M.**
Apartment No. 2, 5 Maple Street
Hopkinton, MA 01748 (US)
Inventor: **McMillian, Ray A.**
14 Brookdale Circle
Shrewsbury, MA 01545 (US)
Inventor: **Lane, David J.**
9 Oriole Drive
Milford, MA 01757 (US)
Inventor: **Collins, Mark L.**
435 Malden Street
Holden, MA 01520 (US)
Inventor: **Aswell, James E.**
35 Lawrence Street
Norfolk, MA 02056 (US)
Inventor: **Rachtchian, Ayoub**
9121 Tulip Grove Road
Gaithersburg, MD 02879 (US)

(74) Representative: **Sheard, Andrew Gregory et al**
Kilburn & Strode
30, John Street
London WC1N 2DD (GB)

EP 0 350 205 B1

Description

This invention relates to detecting bacteria belonging to the genus Campylobacter and more specifically provides nucleic acid probes and compositions along with methods for their use for the specific detection of rRNA or rRNA genes of Campylobacter.

EP-A-0232085 refers to DNA probes that are complementary to part of the rRNA of Campylobacter, that are capable of hybridizing to Campylobacter species but not non-Campylobacter species.

EP-A-0272009 refers to a large number of nucleic acid probes for detecting a variety of non-viral organisms, including probes derived from the rRNA of Campylobacter.

The term "Campylobacter" as used herein, refers to the bacteria classified as such in Bergey's Manual of Systematic Bacteriology, Vol. 1 (N.R. Krieg and J.G. Holt [eds.], 1986, pp.111-118, Williams & Wilkins). Detection of Campylobacter is important in various medical and public health contexts. Campylobacter jejuni and C. coli are the two most important species, causing diarrhoea (Blaser et al., 1979, Ann. Intern, Med. 91:179), and enteritis (G.K. Morris et al., eds, American Society for Microbiology, Washington, D.C.) in humans. Other Campylobacter species have been implicated in causing disease in humans or animals, such as abortion, septicemia and proliferative ileitis. In addition, microorganisms resembling Campylobacter have been isolated from feces of homosexual men (Fennell et al., 1984 J. Infec. Dis. 149:58) and from gastric ulcer biopsies (Kasper et al., 1984 Infection. 12:179).

It is, therefore, an aspect of the present invention to provide a novel assay system capable of rapidly detecting Campylobacter and which is generally applicable to environmental, food or clinical samples.

Campylobacter generally are identified pursuant to a standard laboratory method (Campylobacter, In Washington, J.A. [ed.], Laboratory Procedures in Clinical Microbiology, 2nd Ed., New York, Springer-Verlag, 1985, pp. 215-217).

It is yet another aspect of the present invention to avoid the disadvantages associated with traditional culturing techniques and to employ nucleic acid probes to detect Campylobacter.

It is yet another aspect of the present invention to provide probes which can hybridize to target regions which can be rendered accessible to the probes under normal assay conditions.

While Kohne et al. (1968, Biophysical Journal 8:1104-1118) discuss one method for preparing probes to rRNA sequences they do not provide the teaching necessary to make Campylobacter-specific probes.

Pace and Campbell (1971, Journal of Bacteriology 107:543-547) discuss the homology of ribosomal ribonucleic acids from diverse bacteria species and a hybridization method for quantitating such homology levels. Similarly, Sogin, Sogin, and Woese (1972, Journal of Molecular Evolution 1:173-184) discuss the theoretical and practical aspects of using primary structural characterization of different ribosomal RNA molecules for evaluating phylogenetic relationships.

Fox, Pechman, and Woese (1977, International Journal of Systematic Bacteriology 27:44-57) discuss the comparative cataloging of 16S ribosomal RNAs as an approach to prokaryotic systems. These references, however, fail to relieve the deficiency of Kohne's teaching with respect to Campylobacter.

Rashtchian and Fitts in US-A-4785086 discuss the generation of certain Campylobacter-specific nucleic acid probes targeted at genomic DNA sequences, but the method described would not yield small oligonucleotide probes.

It is yet another aspect to provide small oligonucleotide probes capable of specifically detecting Campylobacter.

Romaniuk et al. (1987, J. Bacteriol. 169:2137-2141), Rashtchian et al. (1987, Current Microbiol. 14:311-317), Lau et al. (1987, System and Appl. Microbiol. 9:231-238), Paster and Dewhirst (1988, Intnl. J. System. Bacteriol. 38:56-62), and Thompson et al. (1988, Intnl. J. System. Bacteriol. 38:190-200) discuss Campylobacter ribosomal RNA gene organization and present 16S rRNA sequences from various Campylobacter. The references, however, fail to identify to probe target regions of most interest.

Romaniuk and Trust (1987, FEMS Microbiol. Lett. 43:331-335), describe an oligonucleotide probe which hybridizes to a region of Campylobacter 16S rRNA and demonstrates its use in identifying stains of Campylobacter by Southern hybridization to electrophoretically-separated restriction fragments of Campylobacter genomic DNA. While useful in this limited context, this probe does not have sufficient specificity to identify Campylobacter in samples containing mixed populations of Campylobacter and non-Campylobacter bacteria.

Ribosomes are of profound importance to all organisms because they serve as the only known means of translating genetic information into cellular proteins, the main structural and catalytic elements of life. A clear manifestation of this importance is the observation that all cells have ribosomes.

Ribosomes contain three distinct RNA molecules which, at least in E. coli, are referred to as 5S, 16S, and 23S rRNAs. These names historically are related to the size of the RNA molecules, as determined by

sedimentation rate. In actuality, however, they vary substantially in size between organisms. Nonetheless, 5S, 16S, and 23S rRNA are commonly used as generic names for the homologous RNA molecules in any bacteria, and this convention will be continued herein.

Hybridization is traditionally understood as the process by which, under predetermined reaction conditions, two partially or completely complementary single-stranded nucleic acids are allowed to come together in an antiparallel fashion to form a double-stranded nucleic acid with specific and stable hydrogen bonds. The stringency of a particular set of hybridization conditions is defined by the base composition of the probe/target duplex, as well as by the level and geometry of mispairing between the two nucleic acids. Stringency may also be governed by such reaction parameters as the concentration and type of ionic species present in the hybridization solution, the type and concentrations of denaturing agents present, and/or the temperature of hybridization. Generally, as hybridization conditions become more stringent, longer probes are preferred if stable hybrids are to be formed. As a corollary, the stringency of the conditions under which a hybridization is to take place (e.g., based on the type of assay to be performed) will largely dictate the preferred probes to be employed. Such relationships are well understood and can be readily manipulated by those skilled in the art. As a general manner, dependent upon probe length, such persons understand stringent conditions to mean approximately 35 °C-65 °C in a salt solution of approximately 0.9 molar.

As used herein, probe(s) refer to synthetic or biologically produced nucleic acids (DNA or RNA) which, by design or selection, contain specific nucleotide sequences that allow them to hybridize under defined predetermined stringencies, specifically (i.e., preferentially) to target nucleic acid sequences.

A target nucleic acid sequence is one to which a particular probe is capable of preferentially hybridizing.

In accordance with the various principles and aspects of the present invention, there are provided nucleic acid probes and probe sets comprising DNA or RNA sequences which, under specific hybridization conditions, are capable of detecting the presence of ribosomal RNA (rRNA) molecules of Campylobacter jejuni, C. coli and C. laridis, but which are not capable, under the same conditions, of detecting the rRNA of other related bacteria which may be present in the test sample. Relevant test samples might include for example, feces, blood, or other body fluids or tissues as well as foods and biological samples or materials from animals.

The probes of the instant invention can be used to identify a number of genetically distinct groups of Campylobacter. These are shown in Table 1 and indicated by boxes surrounding the major groupings. Within these groups, some further subdivision is possible and is indicated by spacing between the subgroups. C. jejuni, C. coli and C. laridis - the Campylobacter species which account for the vast majority of isolates from clinical (stool) specimens and contaminated foods - form a discrete group. These are closely related to one another and distinct (in the pattern and extent of 16S rRNA nucleotide sequence variation) from the other Campylobacter species. Intermixed among the other three Campylobacter groups are a number of non-Campylobacter bacteria including representatives of the genera Wolinella, Bacteroides, Thiovulum and Flexispira (see Table 1).

The probes described herein hybridize principally with the members of the C. jejuni, C. coli and C. laridis group. Because of their overwhelming prevalence compared to other Campylobacter species in the clinical, food and environmental samples of most interest, and because of their genetic distinctness, probes specific for this Campylobacter group are of primary importance.

The present invention also features an assay system for the utilization of these probes, the preferred format of which can advantageously enhance the aforementioned desirable behaviour of the probes. The assay system of the present invention advantageously exhibits the following enhanced performance capabilities with respect to other currently available means for detection of Campylobacter:

a) increased sensitivity; i.e., the ability to detect Campylobacter in a given sample more frequently than currently available methods;

b) potentially significant reductions in assay cost due to the use of inexpensive reagents and reduced labour;

c) accurate identification of even biochemically unusual Campylobacter; and

d) faster results because the test may be performed on uncultured samples which need not be grown further. Accordingly, the preferred test of this invention advantageously takes only one day to provide results.

It has been discovered that other advantages incurred by directing the probes of the present invention against rRNA include the fact that the rRNAs detected constitute a significant component of cellular mass. Although estimates of cellular ribosome content vary, actively growing Campylobacter bacteria may contain upwards of 5.0 x 10E+3 ribosomes per cell, and therefore 5.0 x 10E+3 copies of each of the rRNAs

(present in a 1:1:1 stoichiometry in ribosomes). In contrast, other potential cellular target molecules such as genes or RNA transcripts thereof, are less ideal since they are present in much lower abundance.

A further unexpected advantage is that the rRNAs (and the genes encoding them) appear not to be subject to lateral transfer between contemporary organisms. Thus, the rRNA primary structure provides an organism-specific molecular target, rather than a gene-specific target as would likely be the case, for example, of a plasmid-borne gene or product thereof which may be subject to lateral transmission between contemporary organisms.

Additionally, the present invention provides probes to Campylobacter rRNA target sequences which are capable of distinguishing between a number of Campylobacter (described above). A preferred mixture of two probes also is provided which can hybridize to the rRNA target regions of Campylobacter jejuni, C. coli, and C. laridis, the Campylobacter species most commonly isolated from clinical, food and environmental samples. Advantageously, these same rRNA target sequences are sufficiently different in most non-Campylobacter rRNAs that, under the preferred assay conditions of the present invention, the probe(s) of the present invention hybridize to Campylobacter rRNAs and do not generally hybridize to non-Campylobacter rRNAs. These probe characteristics are defined as inclusivity and exclusivity, respectively. The discovery that probes could be generated with the extraordinary inclusivity and exclusivity characteristics of those of the present invention with respect to Campylobacter was unpredictable and unexpected.

In a particularly preferred embodiment of the invention, an assay method for detecting Campylobacter jejuni, C. coli and C. laridis in stool specimens is provided. The test is rapid, sensitive and non-isotopic, does not require cultivation of bacteria in the sample prior to the hybridization step, and is highly specific for the mentioned Campylobacter.

Brief Description of the Tables

Further understanding of the principles and aspects of the present invention may be made by reference to the tables wherein:

- Table 1 — Shows the relationships among a number of subgroups of Campylobacter species which have been discovered based on analysis of the patterns of sequence variations that exist in Campylobacter 16S rRNAs. This table provides a useful framework for understanding the specificity (inclusivity) of the various probes and probe sets disclosed herein; and
- Table 2a-d — Show alignment of the nucleotide sequences of the probes of the present invention with their nucleotide target sequences of Campylobacter 16S rRNA (using the E. coli position numbering convention, Brosius et al., 1978, Proc. Natl. Acad. Sci. USA 75:4801-4805) along with relevant portions of the 16S rRNAs from other related bacteria including Wollinella, Bacteroides, Flexispira and Thiovulum. The E. coli sequence also is shown for the purpose of identifying the positions of the target regions. RNA sequences are written 5′ to 3′, probe sequences and DNA and written 3′ and 5′. Lower case c in certain of the probes indicates a modified cytosine residue to which a reporter group may or may not be attached depending on the assay form employed; and
- Table 3 — Exemplifies the inclusivity and exclusivity behavior of the preferred probes toward a representative sampling of Campylobacter and non-Campylobacter strains tested by the cytodot procedure.

The first step taken in the development of the probes of the present invention involved identification of regions of 16S rRNA which could potentially serve as target sites for Campylobacter-specific nucleic acid probes. As a practical matter, it is difficult to predict, a priori, which non-Campylobacter organisms might be present in any test sample. Because of the large number of such potential non-Campylobacter bacteria, demonstrating adequate exclusivity for any given probe is extremely difficult and laborious and of unpredictable outcome. A more rigorous criterion was adopted to obviate the need to know, during initial stages of research and development, what non-Campylobacter bacteria might be present in all test samples that ultimately will be screened using the probe. This entailed knowledge of the phylogenetic relationships among Campylobacter and between Campylobacter and other groups of bacteria. Specifically, an operating but previously unproven hypothesis was adopted that the exclusivity criterion could be satisfied by determining that if a particular target region in Campylobacter rRNA, sufficiently different from the homologous region in the rRNA of representative yet close evolutionary relatives of Campylobacter, could be identified, then a probe to such a sequence could be used to distinguish between the Campylobacter and the relatives by hybridization assay. Based on phylogenetic observations, it was then extrapolated that rRNA sequences of more distantly related organisms, even though their actual identity may not necessarily be known, should be predictably different in a particular region of sequence than the aforementioned close

evolutionary relatives of Campylobacter. However, it cannot be predicted, a priori, whether such regions exist or, if they do, where within the rRNA such regions will be located.

As our first step in identifying regions of Campylobacter rRNA which could potentially serve as useful target sites for nucleic acid hybridization probes, nearly complete nucleotide sequences of the 16S rRNAs from a number of Campylobacter species were determined (see Tables 2A-2D). These were selected as representative of the evolutionary breadth of the genus Campylobacter. The nucleotide sequences of various portions of the rRNAs were determined by standard laboratory protocols either by cloning (Maniatis et al., 1982, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 545pp) and sequencing (Maxam & Gilbert, 1977, Proceedings of the National Academy of Science, USA 74:560-564; Sanger et al., 1977, Proceedings of the National Academy of Science, USA 74:5463-5467) the genes which specify the rRNAs, and/or by direct sequencing of the rRNAs themselves using reverse transcriptase (Lane et al., 1985, Proceedings of the National Academy of Science, USA 82:6955-6959).

The nucleotide sequences so obtained were compared to one another and to other available rRNA nucleotide sequences, in particular to those derived from the bacteria shown in Table 1. The preferred regions of sequences shown in Tables 2A-2D were identified as potentially exhibiting useful inclusivity and exclusivity characteristics with respect to these species.

Further experimental testing of each nucleic acid probe was conducted in order to rigorously demonstrate whether the desired characteristics discussed above could indeed be obtained, namely: 1) adequate exclusivity to all, even closely related, non-Campylobacter organisms, 2) useful inclusivity patterns with respect to Campylobacter strains, and 3) accessibility of the target regions under various assay conditions that might actually be employed. Because of the extremely large number of organisms potentially relevant to defining exclusivity (particularly in stool samples where a very large variety and abundance of non-Campylobacter bacteria are found) and inclusivity (on the order of 15 species and biogroups of Campylobacter, and a larger number of "Campylobacter-like organisms") characteristics of test probes, an iterative strategy was adopted to test and refine potential probes. In addition to test panels of cultured organisms, some 75 Campylobacter culture-negative stool specimens were screened using the probes in order to more rigorously demonstrate appropriate exclusivity behavior (Example 1 - Specific). The probes were conveniently synthesized by standard phosphoramidite techniques (Caruthers, M.H. et al. [1983], in Gene Amplification and Analysis, eds. Papas, T.S., Rosenberg, M., Charikjian, J.G., Pub. Elsevier, New York, Vol. 3 pp. 1-26) on an Applied Biosystems instrument.

"Dot blot" analysis, in accordance with well known procedures, was employed to preliminarily test the inclusivity and exclusivity properties of these first generation probes. As is known, dot blot analysis generally involves immobilizing a nucleic acid or a population of nucleic acids on a filter such as nitrocellulose, nylon, or other derivatized membrane which can be readily obtained commercially, specifically for this purpose. Either DNA or RNA can be easily immobilized on such a filter and subsequently can be probed or tested for hybridization under any of a variety of nucleic acid hybridization conditions (i.e. stringencies) with nucleic acid probes of interest. Under stringent conditions, probes whose nucleotide sequences have greater complementarity to the target sequence will exhibit a higher level of hybridization that probes containing less complementarity. For the oligonucleotide probes described herein, (i.e., 30-36 nucleotides) hybridization to rRNA target at 60 °C, for 14-16 hours (in a hybridization solution containing 0.9 M NaCl, 0.12 M Tris-HCl, pH 7.8, 6 mM EDTA, 0.1 M KPO_4, 0.1% SDS, 0.1% pyrophosphate, 0.002% Ficoll®, 0.02% BSA, and 0.002% polyvinylpyrrolidine) followed by three, 15 minute post-hybridization washes at 60 °C to remove unbound probes (in a solution containing 0.03 M NaCl, 0.004 M Tris-HCl, pH 7.8, 0.2 mM EDTA and 0.1% SDS), would be sufficiently stringent to produce the levels of specificity and sensitivity demonstrated in the tables and examples. Techniques are also available in which DNA or RNA present in crude (unpurified) cell lysates can be immobilized without having to first purify the nucleic acid in question (referred to herein as cytodots, see for example Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, a Laboratory Manual). This latter approach significantly decreases the amount of effort required to screen for particular nucleotide sequences which may be present in the nucleic acids of any particular organism and, moreover, is advantageously amenable to the mass screening of large numbers of organisms. Therefore, it is the method of choice for exclusivity and inclusivity screening of potential nucleic acid hybridization probes versus large numbers of organisms.

A list of non-Campylobacter bacteria which exemplify the type of bacteria that may be present in potentially Campylobacter containing samples is given in Example 4. As discussed above, a probe which demonstrates good exclusivity characteristics to such a broad representation of bacteria can reasonably be predicted to behave similarly to a much broader list of more distantly related enteric organisms. This prediction is borne out by the data shown in Example 1 - Specific in which the preferred probes (using a preferred assay format) did not cross-react with any of the non-Campylobacter bacteria present in 75 out of

75 Campylobacter culture-negative stool specimens tested.

Several other considerations also affect optimal design characteristics of a probe. The first is consideration of the geometry of the probe with respect to itself (i.e., intramolecular interactions). It has been discovered that potentially useful target sequences of 16S and 23S rRNAs most often are located in regions that exhibit a substantial possibility for self-complementarity. As a result, probes to these regions must be long enough and of appropriate geometry to complete with the secondary structure of the target molecule itself. Secondly, probes to such (structured) target regions can also exhibit self-complementarity. Because potential interactions between the probe and target sequences are governed by the same types of parameters that govern the intramolecular annealing of the target or probe sequences to themselves, it is possible, particularly under solution hybridization conditions, that self-complementary probes can render themselves inaccessible for hybridization to their target sequences. Thus, one important aspect of the probe design is to minimize such self-complementarity. This necessitates making a compromise between maximum utilization of Campylobacter-specific sequences and acceptable probe geometry.

A second consideration in probe design arises with respect to the inclusivity criterion. The preferred probe will be one which, while displaying appropriate exclusivity behavior, can also hybridize to the rRNA(s) of all desired Campylobacter bacteria. Because the genus Campylobacter itself is comprised of bacteria which exhibit significant phenotypic and genotypic (including, as disclosed below, rRNA) diversity, the design of such an "ideal" probe is greatly complicated. In practice, rather than searching for a single "universal" Campylobacter probe, a set of Campylobacter-specific probes is more preferably sought, each of which exhibits appropriate exclusivity along with a useful level of inclusivity. In aggregate, a preferred set of probes should ideally detect most or all Campylobacter and no non-Campylobacter bacteria. In such a set, for example, one probe may detect all but one or a few important Campylobacter strains, and another probe may hybridize only to those few Campylobacter strains missed by the first probe. Thus, although the probes disclosed below are characterized on an individual basis with respect to inclusivity characteristics, it should be recognized that the concept of "sets" of specific probes as detailed above is preferably considered in determining the importance of individual probes and in constructing assay kits.

The final steps of probe design and analysis ideally comprise testing actual (e.g., food/clinical/environmental) samples and then selecting suitable probes for a final probe set so that the desirable properties are optimized under real assay conditions.

Probes

The foregoing probe selection strategy yielded a number of probes useful for identifying Campylobacter bacteria in samples. As outlined before, Tables 2A-2D give the probe sequences, aligned upon their target sites in the rRNAs of representative Campylobacter strains; Tables 2A through 2D detail the preferred probes.

Table 3 shows the hybridization behavior of the probes versus "cytoblots" of various Campylobacter and non-Campylobacter bacteria. In this experiment the probes were radioactively labelled with Phosphorous-32 for detection and quantitation. Hybridization conditions comprised hybridizing at 60 °C for 14 - 16 hours in the hybridization solution previously described.

It will be readily recognized, however, that as assay formats of higher stringency are employed, the use of longer versions of the probes may become more desirable in order to maintain an equivalent level of sensitivity (hybridization efficiency).

Table 2A describes four probes, targeted at the 124 to 225 region of Campylobacter 16S rRNA (using the E. coli position numbering). The probes designated 345 and 346 have also been identified under the nomenclature AR197 and AR196, respectively. Both were demonstrated to hybridize specifically to rRNA from members of the genus Campylobacter. Under certain sensitive assay formats, probes 345 and 346 can exhibit some undesirable cross-hybridization to some non-Campylobacter present in normal stool specimens, and accordingly these probes are less preferred. Based upon additional sequence analysis, probes 999 and 732 were designed and tested. These are shorter than their "parent" probes (31 and 35 nucleotides long, respectively, versus 50 for both 345 and 346); they also utilize different portions of the Campylobacter-specific sequence positions located in 16S rRNA region 124-225, and thus bear a somewhat different relationship to the thermodynamically favored secondary structure of this target region than probes 345 and 346. Overall, probes 999 and 732 advantageously exhibit equivalent (i.e., full) inclusivity for the target species of Campylobacter (C. jejuni, C. coli and C. laridis) and improved exclusivity with respect to that shown by probes 345 and 346. Significantly less hybridization to non-C. jejuni, C. coli and Campylobacter laridis is exhibited by probes 999 and 732, but this is deemed an acceptable compromise given the improved exclusivity in the presently preferred assay formats (and also because of the relative incidence of

various Campylobacter species in stool - C. jejuni > C. coli > C. laridis >>> all other Campylobacter, as discussed above).

Probes 732 and 999 are, therefore, the most preferred probes described herein because of the just described inclusivity and exclusivity behavior and also because of their apparent sensitivity. The hybridization behavior of probes 732 and 999 is detailed in Table 3 and Examples 1-4. Table 3 shows the behavior of the probes individually in a cytodot format. Examples 1-4 detail the hybridization behavior of the probes used in combination in a most preferred, dual-specific, liquid hybridization format.

Note that in the dual-specific assay format (described in detail below - Example 1), hybridization of both probes is required to produce a positive result. Therefore, the effect of undesirable hybridization (i.e., to non-Campylobacter) by either probe alone may be significantly reduced, if not abolished, in this format.

Table 2B describes two probes, designated 1104 and 1105, targeted at the 391 and 501 region of Campylobacter 16S rRNA (using the E. coli position numbering). Probe 1104 hybridizes to all C. jejuni (5), C. coli (3), and C. laridis (5), which have so far been tested and, in addition, hybridizes to 58 of 62 partially characterized, clinical isolates (mostly C. jejuni). It also hybridizes to a number of, but not all other Campylobacter species. Of the non-Campylobacters tested, only W. curva is detected to any significant extent by probe 1104. Probe 1105 hybridizes to all Campylobacter strains and species so far tested. It also hybridizes to the Bacteroides and Wolinella strains shown in Tables 1 and 2, but not to the enteric strains, E. coli, S. typhimurium, etc. The hybridization behavior of probe 1105 or derivatives thereof would potentially make it a most useful "broad-specificity" probe for the identification/detection of the entire group of Campylobacter and "relatives" shown in Table 1. It is of note that this entire grouping is, by a variety of genetic and biochemical criteria, quite distinct from all other bacteria. A probe or probe set useful for detecting the presence of any member of the group in a natural (e.g. clinical, food or environmental sample) would be a potentially valuable research tool for studying the occurrence and epidemiology of these still poorly understood bacteria. Since many of the non- C. jejuni, C. coli, and Campylobacter laridis are very difficult to isolate or cultivate, not much really is known about their prevalence in the environment or about their association with disease states in animals and humans. These probes would serve as useful tools in gaining such understanding.

Table 2C describes three probes, designated 1130, 1132 and 1133 which are targeted at the 973 to 1049 region of Campylobacter 16S rRNA (using the E. coli position numbering). All exhibit full inclusivity for the C. jejuni, C. coli and C. laridis strains tested (Table 3) and, in addition, detect all 62 of the clinical isolates. Limited hybridization to a small number of non-jejuni, coli or laridis Campylobacter strains is exhibited by all three probes to this region under the hybridization conditions employed. All show excellent exclusivity behavior and likely will be extremely useful as Campylobacter-specific probes.

Note that probes 1132 and 1133 differ only at one position. This difference reflects the heterogeneity observed among the target Campylobacter at this position.

Analog-C, first mentioned in the legend of Table 2C, is 2'-deoxycytidine which has been modified at the C-4 position with a 1,3-propane diamine side chain (Schulman, L.H. et al. (1981), Nucl. Acids Res. 9, 1203-1217). This compound is converted to a phosphoramidite which can be incorporated into a DNA probe using the solid phase synthetic methods developed by Caruthers (Caruthers, M.H., et al. (1982), in Genetic Engineering, Setlow, A. and Hollaender, J.K. (eds.), Vol. 4, pp 1-17, Plenum Press, New York). This primary amine then can be selectively derivatized with, for example, a biotin or fluorescein ligand which can be used to detect the synthetic oligonucleotide (Rashtchian, A., et al., 1987, Clin. Chem. 33/9, 1526-1530).

Table 2D describes two probes including 351, previously disclosed as Probe AR351. The other, probe 1134, was derived from, and is shorter then probe 351 but make use of the same novel structural element in Campylobacter 16S rRNA. Both 351 and 1134 are targeted at the 1424-1489 region of Campylobacter 16S rRNA. Note in Table 2D that, near the middle of the target sequences for probes 351 and 1134, the Campylobacter rRNAs have a conserved deletion of six nucleotides with respect to the E. coli sequence in this region. The E. coli structure is much more representative of that exhibited by the vast majority of eubacteria and thus renders probe 1134 quite specific for Campylobacter. Additional nucleotide differences vicinal to this deletion among the Campylobacter and between the Campylobacter and Bacteroides, Wolinella, etc., further serve to restrict the hybridization of probes 1134 and 351 to members of the C. jejuni, C. coli and C. laridis group of Campylobacter. While some limited hybridization to a few other Campylobacter is detected on dot blots (Table 3), no hybridization to non-Campylobacter is detectable.

Example 1 - General: A Homopolymer Capture, Dual Probe, Liquid Hybridization Format

Cultures containing Campylobacter and/or non-Campylobacter bacteria are grown in appropriate broth, then the nucleic acids are released by any of a number of appropriate lysis agents (e.g., NaOH, guanidine

salts, detergent, enzymatic treatment, or some combination of the aforementioned). Hybridization is carried out with two different probes or probe sets at least one of which, but not necessarily both, must be specific for the organism to be detected. In this example, the Campylobacter specific "capture" probe 732 is enzymatically tailed with 20-200 deoxyadenosine (dA) residues at its 3'-terminus, and the reporter probe, 999, is labeled either chemically or enzymatically with radioactive phosphorus (P-32) or other small ligand (e.g., fluorescein or biotin, the former being used in this experiment) which is used to detect the captured target molecules.

Generally, following cultivation/enrichment, bacteria present in the test samples are transferred in small aliquots to test tubes. The bacteria are lysed, the capture and detection probes are added, and hybridization is allowed to proceed in an appropriate solution at an appropriate temperature such as described below (Example 1 - Specific). The solution containing the target/probe complex then is brought into contact with a surface containing bound deoxythymidine (dT) homopolymer 15-3000 nucleotides in length, under conditions that will allow hybridization between the dA and dT. In this example, the dT is bound to a plastic "dipstick" which is submerged in the target/probe solution. If Campylobacter ribosomal RNA was present in the test sample, the dA tailed, Campylobacter-specific capture probes would have hybridized to the target rRNA sequences present and, in turn, would be captured onto the dipstick. Unhybridized nucleic acids and cellular debris are washed away as described below, leaving the captured DNA-RNA complex attached to the surface via the dA-dT duplex. The reporter probe also is bound to the dipstick via the chain of interactions - Capture surface-dT: dA-Capture probe:Target:Reporter Probe - only if the correct target nucleic acid is present. The bound, ligand derivatized (e.g., fluoresceinated) reporter probe then is detected by the addition of a ligand binding-enzyme complex (e.g., horseradish peroxidase-conjugated anti-fluorescein antibody, in this example). Following incubation under conditions permitting specific binding of the detection complex, washing to remove non-bound enzyme, addition of chromogenic substrate and subsequent color development (typically 20-30 minutes), and the optional addition of color-termination solution, the developed color is measured colorimetrically. This reading (typically in the range of 0.1 - > 2.0 Absorbance units) is compared to the negative control levels, a threshold or cutoff value is established, and a determination of the "significance" of the experimental levels is made.

Example 1 - Specific

For clinical stool specimens, 1g of the sample was added to 3ml of Campylobacter stool processing buffer (3.25M guanidine thiocyanate, 0.4M Tris-HCl (7.5), 0.08M EDTA, 13% dextran sulfate 5000, 0.325% sarkosyl) and vortexed until the sample was homogenized.

0.70ml of the processed sample was used for each hybridization. The nucleic acid released from each sample was detected by addition of 0.05 ml specific capture and detector probes (containing 1.0 microgram/ml of preferred capture probe 732 and 0.5 microgram/ml of detector probe 999-FITC). A capture dipstick was placed into each test tube (containing bacterial lysate and the specific probes). The contents were incubated in a 37 °C water bath for 60 minutes to enable hybridization of specific capture and reporter probes to target nucleic acids and the capture of these specific DNA/rRNA hybrids to the dipsticks as described above.

After hybridization, the dipsticks were washed by briefly submerging the dipsticks in a wash basin containing enough wash solution to cover the active dT coated part of the dipstick (50 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, and 0.1% Tween® 20, room termperature) for one minute.

The washed dipsticks were removed from the wash basin, blotted dry with absorbent paper, placed into a set of test tubes containing 0.75 ml antibody-enzyme conjugates (anti-fluorescein-horseradish peroxidase diluted in wash buffer), and allow to incubate at room temperature for 20 minutes.

After allowing the antigen-antibody reaction to occur, the dipsticks were removed from the test tubes, washed and blotted in the same manner as described in the preceding two paragraphs. The dipsticks were placed into a set of labeled test tubes containing substrate-chromogen mixtures (urea-peroxide:tetramethyl benzidine [Ventrex, Portland, Maine], 2:1) and allowed to incubate at room temperature for 20 minutes. The dipsticks were then removed and the color development step terminated by the addition of 0.25 ml 4N sulfuric acid. The absorbance of the samples were measured colorimetrically using light of wave length 450 nanometers.

Sample tubes with ≥ 0.1 O.D. values were considered positive for Campylobacter, those with lower absorbance values indicated the absence of Campylobacter. Results from 148 stool specimens tested as above are shown below:

		CULTURE	
		Positive	Negative
Invention Assay	Positive	(Confirmed +) 71	(False +) 0
	Negative	(False -) 2	(Confirmed -) 75

$$\text{Sensitivity} \equiv \frac{\text{(Confirmed +)}}{\text{(Total Culture +)}} \times 100 = \frac{71}{71 + 2} \times 100 = 97\%$$

$$\text{Specificity} \equiv \frac{\text{(Confirmed -)}}{\text{(Total Culture -)}} \times 100 = \frac{75}{75 + 0} \times 100 = 100\%$$

(≡ means "defined as")

Example 2

The above procedures were repeated on negative pooled stools which had been seeded with different concentration of Campylobacter jejuni. The following results were obtained:

Seeded Concentration CFU/ml	Absorbance 450nm
0	0.01
1×10^4	0.13
1×10^5	0.38
1×10^6	0.73
1×10^7	1.50

While the description of the invention has been made with reference to detecting rRNA, it will be readily understood that the probes described herein and probes complementary to those described herein will also be useful to detect the genes (DNA) encoding the rRNA and accordingly. Such probes are encompassed within the present invention and the appended claims.

Example 3

Following the procedures of Example 1, Camyplobacter isolates were "seeded" into negative pooled stool at 10^7 CFU/ml and tested in the preferred liquid format using capture probe 732 and detector probe 999-FITC. Sources for the listed bacterial strains are as given in Table 3. The results obtained were:

ORGANISM	STRAIN	ABSORBANCE 450 NM
C.JEJUNI	33560	1.03
C.JEJUNI	N933	0.99
C.JEJUNI	29428	0.90
C.LARIDIS	UA487	1.05
C.LARIDIS	UA577	1.04
C.LARIDIS	35223	0.89
C.COLI	33559	0.88
C.COLI	84-29	0.83
C.FETUS FETUS	33246	0.12
C.FETUS VENEREALIS	33561	0.08
C.HYOINTESTINALIS	35217	0.10
C.MUCOSALIS	43264	0.09
C.SPUTORUM	33562	0.14
C.CRYAEROPHILIA	43157	0.06
C.PYLORI	43504	0.03

Example 4

Example 3 was repeated except that non-Campylobacter isolates were "seeded" into negative pooled stool at 10^8 CFU/ml and tested in the preferred liquid format using capture probe 732 and detector probe 999-FITC. Sources for the listed bacterial strains are as given in Table 3; in addition: (6) Silliker Laboratories, Chicago, IL. The observed results were as follows:

ORGANISM	STRAIN	SOURCE	ABSORBANCE 450 NM
ACINETOBACTER CALCOACETICUS	19606	(1)	0.01
AEROMONAS HYDROPHILIA	7965	(1)	0.01
ALCALIGENES FAECALIS	8750	(1)	0.01
BACILLUS CEREUS	14579	(1)	0.00
BACTEROIDES FRAGILIS	23745	(1)	0.01
BACTEROIDES GRACILIS	33236	(1)	0.01
BACTEROIDES MELLANINOGENICUS	25845	(1)	0.02
BACTEROIDES THETAIOTAMICRON	27941	(1)	0.03
BACTEROIDES UREOLYTICUS	33387	(1)	0.01
BIFIDOBACTERIUM BIFIDUM	35914	(1)	0.00
CANDIDA ALBICANS	18804	(1)	0.03
CANDIDA GLABRATA	2001	(1)	0.03
CANDIDA STELLATOIDIAE	36232	(1)	0.00
CANDIDA TROPICALIS	750	(1)	0.00
CITROBACTER DIVERSUS	27156	(1)	0.00
CITROBACTER FREUNDII	S135	(6)	0.00
CLOSTRIDIUM DIFFICILE	9689	(1)	0.01
CLOSTRIDIUM PERFRINGENS	3624	(1)	0.01
CLOSTRIDIUM SORDELLII	9714	(1)	0.01
EDWARDSIELLA TARDA	15947	(1)	0.02
ENTEROBACTER AEROGENES	13048	(1)	0.01
ENTEROBACTER AGGLOMERANS	S121 B	(6)	0.01
ENTEROBACTER CLOACAE	S134	(6)	0.03
ESCHERICHIA COLI	12036	(1)	0.01
FUSOBACTERIUM MORTIFERUM	9817	(1)	0.01
HAFNIA ALVEI	29927	(1)	0.01
KLEBSIELLA OXYTOCA	13182	(1)	0.01
KLEBSIELLA PNEUMONIAE	S122 F	(6)	0.02
MORGANELLA MORGANII	25830	(1)	0.00
NEISSERIA GONORRHOEAE	9793	(1)	0.00
PEPTOCOCCUS ASACCHAROLYTICUS	29743	(1)	0.00
PEPTOCOCCUS MAGNUS	29328	(1)	0.03

ORGANISM	STRAIN	SOURCE	ABSORBANCE 450 NM
PEPTOSTREPTOCOCCUS ANAEROBIUS	27337	(1)	0.00
PLESIOMONAS SHIGELLOIDES	14029	(1)	0.00
PROPIONIBACTERIUM ACNES	6919	(1)	0.03
PROTEUS MIRABILIS	IG 3109	(5)	0.01
PROTEUS VULGARIS	13315	(1)	0.01
PROVIDENCIA ALCALIFICIENS	9886	(1)	0.01
PROVIDENCIA RETTGERI	29944	(1)	0.00
PROVIDENCIA STUARTII	29914	(1)	0.04
PSEUDOMONAS AERUGINOSA	IG 928	(5)	0.00
SALMONELLA TYPHIMURIUM	23566	(1)	0.00
SERRATIA MARCESENS	RF 972	(1)	0.01
SHIGELLA DYSENTERIAE	RF 970	(1)	0.00
STAPHYLOCOCCUS AUREUS	12600	(1)	0.00
STREPTOCOCCUS FAECALIS	19433	(1)	0.00
VIBRIO PARAHEMOLYTICUS	17802	(1)	0.02
WOLLINELLA CURVA	33238	(1)	0.00
WOLLINELLA RECTA	35224	(1)	0.00
WOLLINELLA SUCCINOGENES	25943	(1)	0.02
YERSINIA ENTEROCOLITICA	9610	(1)	0.01

TABLE 1: 16S rRNA RELATIONSHIPS AMONG THE CAMPYLOBACTER & "RELATIVES".

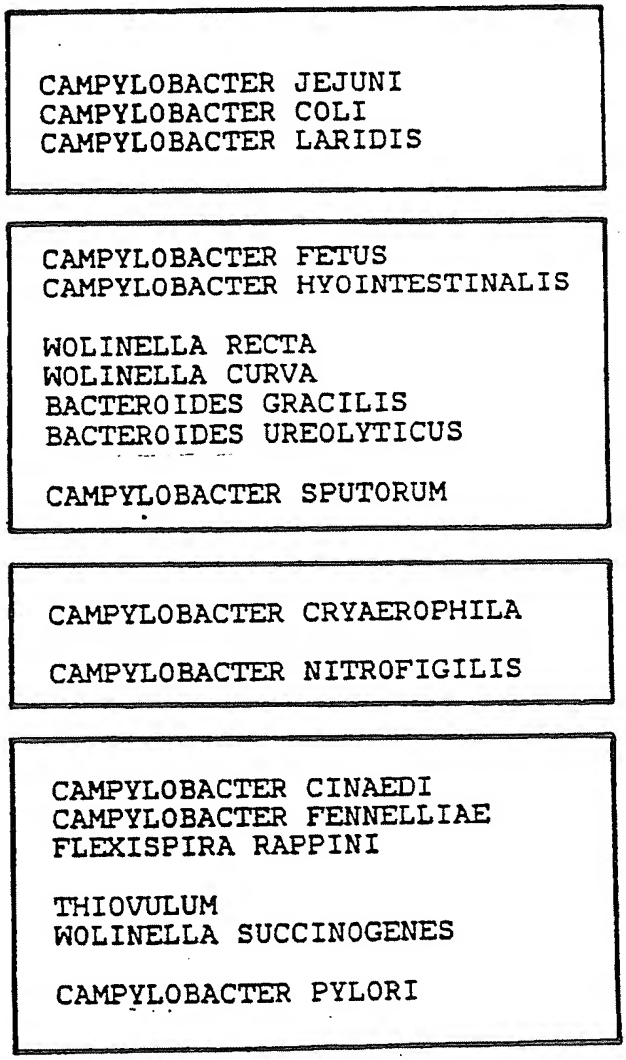

TABLE 2A: CAMPYLOBACTER 16S rRNA PROBE TARGET SITE 124 TO 225.

```
Position #   124                                                                                                  225
             |                                                                                                    |
E. coli      CUGGG-AAACUGCCUGAUGGAGGGGGAUAACUACUGGAAACGGUAGCUAAUACCGCAUAACGUC-------GCAA-------GACCAAAGAGGGGGACCUUCGGGCCUCUUGCCAUC

C.jejuni 1   AUAGUUAAUCUGCCCUACACAAGAGGACAACAGUUGGAAACGACUGCUAAUACUCUAUACUCCUGCUUAACACAAGUUGAGUAGGGAAAG--------UUUUU-------CGGUGUA
C.jejuni 2   AUAGUUAAUCUGCCCUACACAAGAGGACAACAGUUGGAAACGACUGCNNAUACUCUAUNCUCCUGCUUAACACAAGUUGAGUAGGGAAAG--------UUUUU-------CGGUGUA
C.jejuni 3   AUAGUUAAUCUGCCCUACACAAGAGGACAACAGUUGGAAACGACUGCUNAUACUCUAUACUCCUGCUUAACACAAGUUGAGUAGGGAAAG--------UUUUU-------CGGUGUA
C. coli      AUAGUUAAUCUGCCCUACACAAGAGGACAACAGUUGGAAACGACUGCUAUACUCUAUACUCCUGCUUAACACAAGUUGAGUAGGGAHAG--------UUUUU-------CGGUGUA
C. laridis   AUAGUUAAUCUGCCCUACACAAGAGGACAACAGUUGGAAACGACUGCUAAUACUCUAUACUCCUGCUUAACAYAAGUUGAGUAGGGAAAG--------UUUUU-------CGGUGUA

Probe 345                                                                GAGATATGAGGACGAATTGTGTTCAACTCATCCCTTTC--------AAAAA-------GCCACAT
Probe 346      TCAATTAGACGGGATGTGTTCTCCTGTTGTCAACCTTTGCTGACGATTAT
Probe 999                                                  GACGATTATGAGATATGAGGACGAATTGTGT
Probe 732       CAATTAGACGGGATGTGTTCTCCTGTTGTCAACCT

C. fetus f   AUAGUUAAUCUGCCCUACACUGGAGGACAACAGUUAGAAAUGACUGCUMAUACUCCAUACUCCUUCUUAACAUAAGUUAAGUCGGGAAAG--------UUUUU-------CGGUGUA
C. hyoint    AUAGUUAAUCUGCCCUACACUGGAGGACAACAGUUAGAAAUGACUGCUAAUaCUCCAUWCUCCUUCUUAACAUAAGUUAAGUUGGGAAAG--------CCCUUU-------CGGUGUA

C. cryaero   AUAGGUAAUAUGCCUCUUACUAAGGGAUAACAAUUGGAAACGAUUGCUAAUACCUUAUACUCCUUAUUAACCUAAGUUAAUAAGGGAAAGA--------UUUA------UUGGUAAC

Thiovulum    AUAGnUUACAUGCCUnUCGGUCGGGGACAACAGUUGGAAACGACUGCUAAUACCCGAUAUUCCA-------GAAA-------UGGGAAAG--------UUUUU-------CGCCGAA
W. succino   AUAGGUUAUGUGCCCCAUAGUCUGGAAUAGCCACUGGAAACGGUGAUUAAUACCGGAUAUUCCC-------GAGA-------GGGGAAAG--------UUUUU-------CGCUAUG
F. rappini   AUAGGUUAUGUGCCCUUUAGUCUGGCAUAGCCACUGGAAACGGUGAUUAAUACUGGAUNNCUUC-------UACG-------GGGGAAAGG--------UUUU------UCGCUAAA
C. pylori    AUAGGUCAUGUGCCUCUUAGUUUGGGAUAGCCAUUGGAAACGAUGAUUAAUACCAGAUnUCUCC-------UACG-------GGGGAAAGA--------UUUA------UCGCUAAG
```

Legend. 16S rRNA sequences are written in the 5' to 3' direction, Probe sequences are written as DNA, in the 3' to 5' direction. 16S rRNA sequences are aligned with respect to the E. coli sequence which is used as a reference for identifying the Probe Target region. Numbers above the E. coli sequences refer to the number of nucleotide residues counting from the 5' end of the E. coli 16S rRNA sequence. C, A, G, U represent the ribonucleotide bases Cytosine, Adenosine, Guanosine, and Uridine, respectively. W, Y, M, H and N represent uncertain nucleotide assignments: W = A or U, Y = C or U, M = C or A, H = A, C or U, N = A, C, G or U. Lower case letters in the RNA sequences indicate uncertainty in the existence of a nucleotide at that position. Dashes are alignment gaps indicating that no nucleotide is present at that position in the sequence where it appears. Probe 345 is equivalent to probe AR197 and probe 346 is equivalent to probe AR196 as described in EP-A-0232 085 (SN 821,393).
Organism key. E. coli = Escherichia coli sequence from GenBank Data Base; C. jejuni 1 = Campylobacter jejuni strain N941, clinical isolate from Gary Doern, Univ. of Massachusetts Medical Center, Worcester, MA; C. jejuni 2 and C. jejuni 3 are, respectively, strains 29428 and 33560, supplied by the American Type Culture Collection (ATCC); C. coli = Campylobacter coli strain ATCC33559; C. laridis = Campylobacter laridis ATCC35223; C. fetus f = Campylobacter fetus subspecies fetus strain 5396 (Collection of the Institut Pasteur, Paris, France), sequence from P. Romaniuk, pers. comm.; C. hyoint = Campylobacter hyointestinalis ATCC35217; C. cryaero = Campylobacter cryaerophila ATCC43157; Thiovulum cells were isolated from enrichment cultures by D. Stahl (as described in Stahl et al., 1987, Intn'l J. System. Bacteriol., 37:116-122) and sequenced by D. Stahl and D. Lane (reported in Romaniuk et al., 1987, J. Bacteriol. 169:2137-2141); W. succino = Wollinella succinogenes (sequence from Lau et al., 1987, System. & Appl. Microbiol. 9:231-238; F. rappini = Flexispira rappini strain 1937-38264, cells provided by J. Bryner, National Animal Disease Center, Ames, IA; C. pylori = Campylobacter pylori ATCC43504.

EP 0 350 205 B1

TABLE 2B: CAMPYLOBACTER 16S rRNA PROBE TARGET SITE 391 TO 501.

```
             391                                                                                                               501
             |                                                                                                                   |
E. coli      GCAGCCAUGCCGCGUGUAUGAAGAAGGCCUUCGGGUUGUAAAGUACUUUCAGCGGGGAG-GAAGGGAGUAAAGUUAAUACCUUUGCUCAUUGACGUUACCCGCAGAAG-AAGC

C.jejuni 1   GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACUCCUUUUCUUAGGGAA-GAAU------------------------UCUGACGGUACCUAAGGAAU-AAGC
C.jejuni 4   GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACUCCuUUUCUUAGGGAA-GAAU------------------------UCUGACGGUACCUnAGGAAU-AAGC
C.jejuni 5   GCAGCNACGCCGCGUGGAGGAUGACACUUUUCGGAGCG-AAACUNCUUUUCUUAGGGAA-GAAU------------------------UCUNNNGGUACCUAAGGAAU-AAGC
C.jejuni 6   GCAGCNACGCCGCGUGGAGGAUNACACUUUUNGGAGCGUAAACUCCNUUUCUUAGGGAA-GAAU------------------------UCUNNNGGUACCUNAGGAAU-NA..
C. coli      GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACUCCUUUUCUUAGGGAA-GAAU------------------------UCUGACGGUACCUAAGGAAU-AAGC
C. coli 2    GCAGCNACGCCGCGUGGAGGAUGACNCUUUUAGGAGCGUAAACUNNNUUUCUUAGGGAA-GAAU------------------------UCUGANGGUACCUNAGGAAU-AAGC
C. laridis   GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACYCCYUUUCUUAGGGAA-GAAU------------------------UCUGACGGUACCUAAGGAAU-AAGC
C.laridis2   GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACUCNUUUUCUUAGGGAA-GAAU------------------------UCUGNNGGUACCUNAGGAAU-AAGC

probe 1104                                                  TcTCCCTT-CTTA------------------------AGACTGCCATGGATTCCTTA-TTc
probe 1105      TcTGCGGCGCACCTCCTACTGTGAAAAGCCTCGCATTTGAGGAAAAGc

C. fetus 1   GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACUCCNUUUGUUAGGGAA-GAAC------------------------CAUGACGGUACCUAACGAAU-AAGC
C. fetus 2   GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACUMCNUUUGUUAGGGAA-GAAC------------------------CAUGACGGUACCUNASGAAU-AAGC
C. hyoint    GCAGCAACGCCGCGUGGAGGAUGACUCUNUUCGGAGCGUAAACUNCNUUUGUUGGGGAA-GAAC------------------------CAUGACGGUACCCAACGAAU-AAGC
C. concisus  GCAGCAACGCCGCGUGGANGAUGACACUUUUCGGAGCGUAAACUCCNUUUGUWAGGGAA-GAAU------------------------NAUGNCGGUACCUNACGAAU-AAGC
W. curva     GCAGCAACGCCGCGUGGAGGAUGACNCUUUUCGGAGCGUAAACUCUNUUUCUNGGGGAA-GAAA------------------------UUUGACGGUNCCCNAGGAAU-AAGC
W. recta     GCAGCANCGCCGCGUGGAGGAUGACACUNNNNGGAGCGUNAACUNNNNNNNNUNNGGAAAGAAU------------------------NNUGACGGUACCCAAGGAAU-AAGC
B. gracilis  GCAGCANCGCCGCGUGGAGGAUGACACUNUUCGGAGCGUAAACUCNNNUUGUUAGGGAA-GAAU------------------------AAUGACGGUACCUAACGAAU-AAGC
B. ureolyt   GCAGCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUNAACUCCUUUUAUCAGGAAA-GAAU------------------------AAUGACGGUACCUNAUGAAU-AAGC
C.sputorum1  GCAGCAACGCCGCGUGGAGGAUGACNCUUUUCGGAGCGUAAACNCCNNUUCUUUGGGAA-GAAU------------------------AAUGACGGUACCAAAGGAAU-AAGC
C.sputorum2  GCANCAACGCCGCGUGGAGGAUGACACUUUUCGGAGCGUAAACUCCNYUUCUUUGGGAA-GAAU------------------------AAUGACGGUACSAAAGGAAU-AAGC

C. cryaero   GCAGCAACGCCGCGUGGAGGAUGACACAUUUCGGUGCGUNAACUCCYUUUAUAUGAGAA-GAUA------------------------AUGACGGUAUUAUAUGAAU-AAGC
C. cryaero2  GCAGnnACGCCGCGUGGAGGAUnACACAUUUCGGUGCGUnAACUnCUnUUAUAUGAGAA-GAUA------------------------AUAA...
C. nitrofig  GCAGCAACGCCGCGUGGAGGAUGACACAUUUCGGUGCGUAAACUnCnUUUAUAUAGGAA-GAUA------------------------AUGA...

Thiovulum    GCAGCAACGCCGCGUGGAGGAUGACGCAUUUCGGUGUGUAAACUCCUUUUUUCGGAGAA-GaUU------------------------aUGACGGUAUCCGAAGAAU-AAGC
W. succino   GCAGCaACGCCGCGUGGAGGAUGAAGGUCUUCGGAUUGUAAACUCCUUUUCUAAGAGAA-GAUU------------------------AUGACGGUAUCUUAGGAAU-AAGC
C. cinaedi   GCAGCAACGCCGCGUGGAGGAUAAAGGUnnnAGGAUUGUAAACUnnnnUGGUAAGAGAA-GAUU------------------------AUAA...
C. fennell   GCAGCAACGCCGCGUGGAGGAUGAAGGUnnAAGGAUUGUAAACUnCnUUUGUAAGAGAA-GAUU------------------------AUAA...
F. rappini   GCAGCAACGCCGCGUGGAGGAUGAAGGUUUUAGGAUUGUAAACUCCUUUUGUUAGAGAA-GAUU------------------------AUGACGGUAUCUAANGAAN-AAGN
C. pylori    GCAGCAACGCCGCGUGGAGGAUGAAGGUUUUAGGAUUGUAAACUCCYUUUGUUAGAGAA-GAUA------------------------AUGACGGUAUCUAACGAAU-AAGC
```

Legend. Organisms key and sequence notation are as in Table 2A, with the following additions: C. jejuni 5 = Campylobacter jejuni strain UVIC (pers. comm. from P. Romaniuk, Univ. of British Columbia, Vancouver, B.C.); C. jejuni 6 = Campylobacter jejuni strain NCTC11392 (P. Romaniuk); C. coli 2 = Campylobacter coli strain NCTC11366 (P. Romaniuk); C. laridis 2 = Campylobacter laridis ATCC35221 (an amalgam of sequences from Romaniuk et al., 1987, and Thompson et al., 1988, by D. Lane); C. fetus 1 = an amalgam of reverse transcription sequences from Campylobacter fetus subspecies fetus strains VPI H641 (Paster & Dewhirst, 1988), ATCC27374 (Thompson et al., 1988), and CIP5396 (Romaniuk et al., 1987) by D. Lane; C. fetus 2 = an amalgam of Campylobacter fetus subspecies venerialis strain ATCC19438 (Thompson et al., 1988) and an unidentified strain (P. Romaniuk, Pers. Comm.); C. concisus = a Campylobacter concisus amalgam derived from strains FDC (Forsyth Dental Center)288, FDC484 (B. Paster & F. Dewhirst, Forsyth Dental Center, Boston, MA, Pers. Comm) and strain ATCC13086 (Thompson et al., 1988); W. curva = Wollinella curva ATCC35224; W. recta = Wollinella recta ATCC33238; B. gracilis = Bacteroides gracilis ATCC33236; B. ureolyt = Bacteroides ureolyticus ATCC33387; C.sputorum1 = an amalgam of Campylobacter sputorum subspecies bubulus derived from strain ATCC33562 (= C.sputorum, as defined in TABLE 2A) and strain ATCC33491; C.sputorum2 = an amalgam of Campylobacter sputorum subspecies sputorum derived from strains S-17 (Thompson et al., 1988) and Romaniuk et al., Pers. Comm.); C. cryaero2 = Campylobacter cryaerophila ATCC11885 (Thompson et al., 1988); C. cinaedi = Campylobacter cinaedi ATCC35683 (Thompson et al., 1988); C. fennell = Campylobacter fennelliae ATCC35684 (Thompson et al., 1988); S = C or G uncertainty in nucleotide assignment.

TABLE 2C: CAMPYLOBACTER 16S rRNA PROBE TARGET SITE 973 TO 1049.

Position #	973 ... 1049
E. coli	GAAGAACCUUACCUGGUCUUGACAUCCACGGAAGUUUUCAGAGAUGAGAAUGUG-CC--UUCG--GGAACCGUGAGACAGGU
C.jejuni 1	GAAGAACCUUACCUGGGCUUGAUAUCCUAAGAACCUUWUAGAGAUAWGAGGGUG-CUAGCUUGCUAGAACUUAGAGACAGGU
C.jejuni 2	GAAGAACCUUACCUGGNYUUGAUAUCCUAAGAACCUUUUAGAGAUAAGAGGGUG-CUAGCUUGCUAGAACUUAGAGACAGGU
C.jejuni 3	GAAGAACCUUACCUGGNNNUGAUAUCCUAAGAACCUUAUAGAGAUAUGAGGGUG-CUAGCUUGCUAGAACUUAGAGACAGGU
C. coli	GAAGAACCUUACCUGGGCUUGAUAUCCUAAGAACCUUUUAGAGAUAAGAGGGUG-CUAGCUUGCUAGAACUUAGAGACAGGU
C. laridis	GAAGAACCUUACCUGGGCUUGAUAUCCUAAGAACCUUAUAGAGAUAUGAGGGUG-CUAGCUUGCUAGAACUUAGAGACAGGU
Probe 1132	TcCTATACTCCCAC-GATCGAACGATCTTGAATCTCTGc
Probe 1133	TcCTATTCTCCCAC-GATCGAACGATCTTGAATCTCTGc
Probe 1136	
Probe 1130	TcTCTTGGAATGGACCCGAACTATAGGATTCTTGGAAc
C. hyoint	GAAGAACCUUACCUGGGCUUGAUAUCCUAAUAACAUCUUAGAGAUAAGAUNGUG-CUUGUUUACAAGAAAUUAGUGACAGGU
C.sputorum	GAAGAACCUUACCCGGACUUGAUAUCUAACAAAUCAUCUAGAGAUAGAAGAGUG-UCUGCUUGCAGAAAUGUUAAGACAGGU
C. cryaero	GAAGAACCUUACCUKGACUUGACAUAGUAAGAACUUUCUAGAGAUAGAUUGGUG-UCUGCUUGCAGUWACUUAUAUACAGGU
Thiovulum	GAAAAACCUUACCUACCCUUGACAUUGUAAGAAUUUUGCAGAGAUGUGAAAGUG-CU---UCG--GGAACUUGAAAACAGGU
W. succino	GAAGAACCUUACCUGGGCuUGACAUUGAuaGAAUCCUAUAGAGAUAUGGAGUG-CCAGUUUACUGGAGCUUGAaaaCAGGU
F. rappini	GAAGAACCUUACCUAGGCUUGACAUUGAUAGAAUCCGCUAGAGAUAGUGGAGUG-CUGGCUUGCCAGAGCUUGAAAACAGGU
C. pylori	GAAGAACCUUACCUAGGCUUGACAUUGAGAGAAUCCGCUAGAAAUAGUGGAGUGUCUAGCUUGCUAGACCUUGAAAACAGGU

Legend. Organism key and sequence notation are as in Tables 2A & 2B, with the following additions: C. sputorum = Campylobacter sputorum subspecies bubulus ATCC33562. Certain of the probe oligonucleotides have a lower case C (c) at one or both ends. This designates an "analog c" residue to which various "detection" ligands (e.g. biotin, fluorescein) can be readily attached. They do not otherwise affect the behavior of the probes. In appending an analog c to the 3′ ends during direct synthesis, a T residue often is coupled first as a convenience in the systhesis. Again the T residue is not a necessary part of the probe "proper" in that it does not necessarily participate in the hybridization of the probe to its target sequence.

TABLE 2D: CAMPYLOBACTER 16S rRNA PROBE TARGET SITE 1424 TO 1489.

Position #	1424	1489
E. coli	UUGCAAAAGAAGUAGGUAGCUUAACCUUCGGGAG-GG-CGCUUACCACUUUGUGAUUC--AUGACUGGGG	
C.jejuni 1	UUUCACUCGAAGCCGGAAUACUAAACU------A-GU-UACCGUCCACAGUGGAAUCACCGGCGCUGGGG	
C.jejuni 2	UUUCACUNGAAGCCGGAAUACUAAACU------A-GU-UACCGUCCACAGUGGAA...	
C.jejuni 3	UUUCACUCGAAGCCGGAAUACUAAACU------N-GU-UACCGUCCACAGUGGAA...	
C.jejuni 4	UUUCACUCGAAGCCGGAAUACUAAACU------A-GU-UACCGUCCACAGUGGAAUcA--GCGACUGGGG	
C. coli	UUUCACUCGAAGCCGGAAUACUAAACU------A-GU-UACCGUCC...	
C. laridis	UUUCACUCGAAGCCGGAAUACUAAACU------A-GU-UACCGUCCACAGUGGAA...	
Probe 351	TGAGCTTCGGCCTTATGATTTGA------T-CA-ATGGCAGGTGTCACCTTAGTGGCCGC	
Probe 1134	TcGTGAGCTTCGGCCTTATGATTTGA------T-CA-ATGGCAGGc	
C. hyoint	UUUCACUCGAAGUCGGAAUGCUWAAUU------A(CG)UACCGCCCACAGUGGAAU...	
C.sputorum	UUUCACUCGAAGCCCAAAUACCAAAUU------G-GU-UANGGUCCACAGUGGAA...	
C. cryaero	ACUCAUUCGAAGCGGGGAUGCUAAAAU------A-GC-UACCUUCCACAGUGGAUUUG--GCU...	
W. succino	AUUCGCCUUAAGCCGGGACGCUAAACU------G-GC-UACCGUCCaCGGCGGAUGCA--GCGACnGGGG	
F. rappini	AUUCGCCUUAAGUCGGGAUACCMAAUU------G-GU-...	
C. pylori	GUUUGCCUUAAGUCAGGAUGCUAAAUU------G-GC-UNCUGCCCACGGCACACA...	

Legend. Organims key and sequence notation are as in Tables 2A, 2B & 2C with the following additions: C.jejuni 4 = CAMPMERG, an amalgam of 16S rRNA partial reverse transcription sequences from a number of C. jejuni strains (Lau et al., Systematic & Appl. Microbiol., 1987, 9:231-238). The parentheses around the CG dinucleotide in the C. hyoint sequence indicates that the bands identifying these two nucleotides migrated somewhat anomalously on sequencing gels.

EP 0 350 205 B1

TABLE 3. CYTODOT HYBRIDIZATIONS

Genus, Species	Strain	Source	Hybridization Probe 732	Probe 999	Probe 1104	Probe 1105	Probe 1130	Probe 1132	Probe 1133	Probe 1134
C. jejuni	29428	(1)	+	+	+	+	+	+	+	+
C. jejuni	33560	(1)	+	+	+	+	+	+	+	+
C. jejuni	N933	(2)	+	+	+	+	+	+	+	+
C. jejuni	N941	(2)	+	+	+	+	+	+	+	+
C. jejuni	R1227	(2)	+	+	+	+	+	+	+	+
C. coli	33559	(1)	+	+	+	+	+	+	+	+
C. coli	84-29	(3)	+	+	+	+	+	+	+	+
C. coli	P1077	(2)	+	+	+	+	+	+	+	+
C. laridis	35223	(1)	+	+	+	+	+	+	+	+
C. laridis	11253	(1)	+	+	+	+	+	+	+	+
C. laridis	UA487	(4)	+	+	+	+	+	+	+	+
C. laridis	UA577	(4)	+	+	+	+	+	+	+	+
C. laridis	UA603	(4)	+	+	+	+	+	+	+	+
C. spp. (62 isolates)	clinical	(2,3,4)	62+	62+	58+	62+	62+	62+	62+	62+
C. fetus fetus	33246	(1)	+	+	-	+	+	+	+	-
C. fetus venerealis	33561	(1)	+	-	-	+	+	+	+	-
C. hyointestinalis	35217	(1)	+	-	-	+	-	-	-	-
C. hyointestinalis	UA625	(4)	+	+	+	+	+	+	+	-

TABLE 3. CYTODOT HYBRIDIZATIONS (cont'd)

Genus, Species	Strain	Source	Hybridization Probe 732	Probe 999	Probe 1104	Probe 1105	Probe 1130	Probe 1132	Probe 1133	Probe 1134
C. concisus	33237	(1)	+	+	+	+	-	-	-	+
C. mucosalis	43264	(1)	+	-	-	+	-	-	-	-
C. sputorum	33562	(1)	-	-	+	+	-	-	-	-
C. cinaedi	35683	(1)	-	+	+	+	+	+	-	+
C. fennelliae	35684	(1)	-	-	+	+	-	-	-	+
C. pylori	43504	(1)	-	-	-	+	-	-	-	-
C. cryaerophila	43157	(1)	-	-	-	+	-	-	-	-
C. nitrofigilis	33309	(1)	-	-	-	+	-	-	-	-
C. fecalis	UA689	(4)	+	-	+	+	+	+	+	-
C. fecalis	33790	(1)	-	+	-	+	-	-	-	-
Bacteriodes gracilis	33236	(1)	-	-	-	+	-	-	-	-
B. ureolyticus	33387	(1)	+	-	-	+	-	-	-	-
Wolinella recta	33238	(1)	+	-	-	+	-	-	-	-
W. curva	35224	(1)	+	-	+	+	-	-	-	-
W. succinogenes	25943	(1)	-	-	-	+	+	-	-	-
Pseudomonas aeruginosa	IG928	(5)	-	-	-	-	-	-	-	-
Escherichia coli	N99	(5)	-	-	-	-	-	-	-	-
Salmonella typhimurium	23566	(1)	-	-	-	-	-	-	-	-

EP 0 350 205 B1

TABLE 3. CYTODOT HYBRIDIZATIONS (cont'd)

Legend: Hybridization with cytodots of each strain are approximated for each probe as positive (+) or negative (-). Positive signals vary from very strong (i.e., for C. jejuni, C. coli and C. laridis strains) to variable (e.g., non-jejuni, coli or laridis Campylobacter strains) or quite weak (i.e., for the non-Campylobacter). The 62 clinical isolates have not all been typed to the species level, presumably most are C. jejuni, C. coli or C. laridis strains.

Sources for the listed bacterial strains are as follows:

(1) American Type Culture Collection, Rockville, MD
(2) Gary Doern, University of Mass. Med. Cntr., Worcester, MA
(3) M.J. Blaser, VA Medical Center, Denver, CO
(4) Tanya Sanderos, Vermont Dept. of Health, Burlington, VT
(5) GTS in-house isolate

Claims

1. A nucleic acid probe that can hybridize to rRNA or rRNA genes of Campylobacter jejuni, Campylobacter coli and Campylobacter laridis, and does not hybridize to rRNA or rRNA genes of Pseudomonas

aeruginosa, E. coli or Salmonella typhimurium, wherein the probe is complementary or identical to at least 95% of at least any ten consecutive nucleotides from the region 1013 to 1049 or region 1424 to 1489 (using the E. coli position numbering convention) of the 16S rRNA of Campylobacter jejuni, and any combination thereof.

2. A nucleic acid probe as claimed in claim 1 comprising a sequence complementary to the region 1013 to 1049 or 1424 to 1489 (of the 16S rRNA of Campylobacter jejuni)

3. A nucleic acid probe as claimed in claim 1 or 2 which is the probe 1132, probe 1133, probe 351 or probe 1134, or the complementary sequence thereto.

4. A set of nucleic acid probes for detecting Campylobacter in a sample comprising at least two nucleic acid probes as defined in claim 1.

5. A set of probes according to claim 4 comprising a first probe which is probe 1132 or probe 1133, and a second probe which is probe 351 or probe 1134.

6. A set of probes according to claim 4 or 5 comprising a first probe which is probe 1132 and a second probe which is probe 1133.

7. A method of detecting the presence of Campylobacter in a sample, the method comprising:
(a) contacting a nucleic acid probe as defined in any of claims 1 to 3 with the sample under conditions that allow the probe to hybridize to rRNA of Campylobacter jejuni, Campylobacter coli and/or Campylobacter laridis, if present in the sample, to form hybrid nucleic acid complexes; and
(b) detecting the hybrid complexes as an indication of the presence of Campylobacter jejuni, Campylobacter coli and/or Campylobacter laridis in the sample.

8. A method as claimed in claim 7 wherein the nucleic acid probe is capable of hybridizing under hybridizing conditions to a probe which is 1132, 1133, 351 or 1134 and any complementary probe.

9. An assay kit for detecting Campylobacter jejuni, Campylobactor coli and Campylobacter laridis comprising a nucleic acid probe as claimed in any of claims 1 to 3 packaged in at least one container, and optionally instructions for utilizing the nucleic acid probe for detecting the presence of at least one member of the group Campylobacter jejuni, Campylobactor coli and/or Campylobacter laridis in a sample.

Patentansprüche

1. Nucleinsäuresonde, die mit rRNA oder rRNA-Genen von Campylobacter jejuni, Campylobacter coli und Campylobacter laridis hybridisieren kann und nicht mit rRNA oder rRNA-Genen von Pseudomonas aeruginosa, E. coli oder Salmonella typhimurium hybridisiert, wobei die Sonde komplementär zu oder identisch mit mindestens 95 % von mindestens 10 beliebigen aufeinanderfolgenden Nucleotiden aus dem Bereich 1013 bis 1049 oder dem Bereich 1424 bis 1489 (unter Verwendung der üblichen E. coli-Positionsnumerierung) der 16S-rRNA von Campylobacter jejuni ist, und beliebige Kombinationen davon.

2. Nucleinsäuresonde nach Anspruch 1, umfassend eine Sequenz, die komplementär zum Bereich 1013 bis 1049 oder 1424 bis 1489 (der 16S-rRNA von Campylobacter jejuni) ist.

3. Nucleinsäuresonde nach Anspruch 1 oder 2, nämlich Sonde 1132, Sonde 1133, Sonde 351 oder Sonde 1134 oder die dazu komplementäre Sequenz.

4. Satz von Nucleinsäuresonden zum Nachweis von Campylobacter in einer Probe, umfassend mindestens zwei Nucleinsäuresonden gemäß der Definition in Anspruch 1.

5. Sondensatz nach Anspruch 4, umfassend eine erste Sonde, nämlich Sonde 1132 oder Sonde 1133, und eine zweite Sonde, nämlich Sonde 351 oder Sonde 1134.

6. Sondensatz nach Anspruch 4 oder 5, umfassend eine erste Sonde, nämlich Sonde 1132, und eine zweite Sonde, nämlich Sonde 1133.

7. Verfahren zum Nachweis der Gegenwart von Campylobacter in einer Probe, umfassend:
(a) Inkontaktbringen einer Nucleinsäuresonde gemäß der Definition in einem der Ansprüche 1 bis 3 mit der Probe unter Bedingungen, die eine Hybridisierung der Sonde mit rRNA von Campylobacter jejuni, Campylobacter coli und/oder Campylobacter laridis, falls diese in der Probe vorhanden sind, erlauben, wobei Hybrid-Nucleinsäurekomplexe entstehen; und
(b) Nachweis der Hybridkomplexe als einen Hinweis auf die Gegenwart von Campylobacter jejuni, Campylobacter coli und/oder Campylobacter laridis in der Probe.

8. Verfahren nach Anspruch 7, wobei die Nucleinsäuresonde unter Hybridisierungsbedingungen mit einer Sonde hybridisieren kann, nämlich 1132, 1133, 351 oder 1134, und einer beliebigen komplementären Sonde.

9. Testkit zum Nachweis von Campylobacter jejuni, Campylobacter coli und Campylobacter laridis, umfassend eine Nucleinsäuresonde nach einem der Ansprüche 1 bis 3, verpackt in mindestens einem Behälter, und gegebenenfalls Instruktionen zur Verwendung der Nucleinsäuresonde zum Nachweis der Gegenwart von mindestens einem Mitglied der Gruppe Campylobacter jejuni, Campylobacter coli und/oder Campylobacter laridis in einer Probe.

Revendications

1. Sonde d'acide nucléique, qui peut s'hybrider avec un ARNr ou des gènes d'ARNr, de Campylobacter jejuni, Campylobacter coli et Campylobacter laridis, et qui ne s'hybride pas avec un ARNr ou des gènes d'ARNr de Pseudomonas aeruginosa E. coli ou Salmonella typhimurium, dans laquelle la sonde est complémentaire ou identique à au moins 95% d'au moins dix nucléotides consécutifs de la région 1013 à 1049 ou de la région 1424 à 1489 (en utilisant la convention de numérotation de position de E. coli) de l'ARNr 16S de Campylobacter jejuni, et de n'importe laquelle de leurs combinaisons.

2. Sonde d'acide nucléique selon la revendication 1, comprenant une séquence complémentaire à la région 1013 à 1049 ou 1424 à 1489 (de l'ARNr 16S de Campylobacter jejuni).

3. Sonde d'acide nucléique selon la revendication 1 ou 2, qui est la sonde 1132, la sonde 1133, la sonde 351 ou la sonde 1134, ou la séquence complémentaire de celles-ci.

4. Jeu de sondes d'acide nucléique pour la détection de Campylobacter dans un échantillon, comprenant au moins deux sondes d'acide nucléique telles que définies dans la revendication 1.

5. Jeu de sondes selon la revendication 4, comprenant une première sonde qui est la sonde 1132 ou la sonde 1133, et une deuxième sonde qui est la sonde 351 ou la sonde 1134.

6. Jeu de sondes selon la revendication 4 ou 5, comprenant une première sonde qui est la sonde 1132 et une deuxième sonde qui est la sonde 1133.

7. Procédé de détection de la présence de Campylobacter dans un échantillon, qui comprend :
(a) la mise en contact d'une sonde d'acide nucléique telle que définie dans l'une quelconque des revendications 1 à 3 avec l'échantillon dans des conditions qui permettent à la sonde de s'hybrider à l'ARNr de Campylobacter jejuni, Campylobacter coli et/ou campylobacter laridis, s'ils sont présents dans l'échantillon, pour former des complexes d'acides nucléiques hybrides ; et
(b) la détection des complexes hybrides comme une indication de la présence de Campylobacter jejuni, Campylobacter coli et/ou Campylobacter laridis dans l'échantillon.

8. Procédé selon la revendication 7, dans lequel la sonde d'acide nucléique est capable d'hybridation dans des conditions d'hybridation à une sonde qui est 1132, 1133, 351 ou 1134 et toute sonde complémentaire.

9. Trousse d'essai pour la détection de Campylobacter jejuni, Campylobacter coli et Campylobacter laridis, comprenant une sonde d'acide nucléique selon l'une quelconque des revendications 1 à 3 conditionnée dans au moins un récipient, et éventuellement des instructions d'utilisation de la sonde d'acide nucléique pour détecter la présence d'au moins un élément du groupe Campylobacter jejuni, Campylobacter coli et/ou Campylobacter laridis dans un échantillon.